

This listing of claims will replace all prior versions and listings of claims in the application. Please amend claims 1, 90-91, 97, 102-105, 108, and 111 as follows:

- 1. (currently amended) A composition for macerating whole tissue, wherein the whole tissue is not blood, releasing nucleic acids from a biological sample comprising: at least one cationic surfactant, at least one protease, and a buffer, and a salt at a concentration of about 550 mM or less.
- 2. (original) The composition of claim 1, wherein the at least one cationic surfactant is protonated under the conditions used.
- 3. (original) The composition of claim 1, wherein the at least one cationic surfactant has the structure:

$$R_3$$
  $R_2$   $R_3$   $R_4$   $R_4$ 

wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are independently selected from the group consisting of: –H; an alkyl group comprising between one and twenty carbon atoms; and an aryl group comprising between six to twenty-six carbon atoms.

4. (original) The composition of claim 3, wherein the cationic surfactant is an alkyltrimethyl ammonium salt, where R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are methyl groups, and R<sub>4</sub> is an alkyl group comprising 6, 8, 10, 12, 14, 16, or 18 carbon atoms.

- (original) The composition of claim 4, where the cation of the alkyltrimethyl ammonium salt is selected from the group consisting of cetyltrimethylammonium, hexadecyltrimethylammonium, tetradecyltrimethylammonium, dodecyltrimethylammonium, and lauryl trimethylammonium.
- 6. (original) The composition of claim 4, where the anion (X<sup>-</sup>) of the alkyltrimethyl ammonium salt is selected from the group including bromide, chloride, iodide, hydroxide, nitrate, sulfate, phosphate, formate, acetate, propionate, oxalate, malonate, succinate, or citrate.
- 7. (original) The composition of claim 3, wherein the at least one cationic surfactant is a benzyldimethyl-*n*-alkylammonium salt, where R<sub>1</sub> and R<sub>2</sub> are methyl groups, R<sub>3</sub> is an aryl group comprising six carbon atoms, and R<sub>4</sub> is an alkyl group comprising 6, 8, 10, 12, 14, 16, or 18 carbon atoms.
- 8. (original) The composition of claim 7, where the anion of the benzyldimethyl-*n*-alkylammonium salt is selected from the group consisting of bromide, chloride, iodide, hydroxide, nitrate, sulfate, phosphate, formate, acetate, propionate, oxalate, malonate, succinate, and citrate.
- 9. (original) The composition of claim 1, wherein the at least one protease is selected from the group consisting of subtilisins, subtilases, and alkaline serine proteases.
- 10. (original) The composition of claim 9, wherein the at least one protease is selected from the group consisting of Proteinase K, Proteinase R, Proteinase T,

- Subtilisin DY, an alkaline serine protease from *Streptomyces griseus* or *Bacillus licheniformis,* Dispase, subtilisin Carlsberg, subtilopeptidase A, and thermolysin.
- 11. (original) The composition of claim 9, wherein the protease is Proteinase K.
- 12. (original) The composition of claim 9, wherein the protease is thermolysin or a thermostable protease.
- 13. (original) The composition of claim 12, wherein the protease is from *Thermus* Rt41A or *Bacillus thermoproteolyticus rokko*.
- 14. (original) The composition of claim 1, further comprising calcium chloride.
- 15. (original) The composition of claim 1, wherein the buffer maintains the pH between pH 7 and pH 9.
- 16. (original) The composition of claim 1, wherein the buffer maintains the pH between pH 5 and pH 7.
- 17. (original) The composition of claim 1, further comprising a ribonuclease inhibitor.
- 18. (original) The composition of claim 17, where in the at least one ribonuclease inhibitor comprises aurintricarboxylic acid, vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenyhlic acid), zinc sulfate, bromopyruvic acid, formamide, copper, or zinc.

- 19. (original) The composition of claim 18, wherein the at least one ribonuclease inhibitor comprises aurintricarboxylic acid.
- 20. (original) The composition of claim 1, wherein the cationic surfactant is cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTACI), hexadecyltrimethylammonium bromide, or hexadecyltrimethylammonium chloride; the protease is Proteinase K; the buffer maintains the pH between pH 5 and pH 7; and further comprising aurintricarboxylic acid.
- 21. (original) The composition of claim 20, further comprising at least one solubilizing agent for enhancing the solubility or permeability of the sample.
- (original) The composition of claim 21, wherein the solubilizing agent is 1-methyl2 pyrolidinone, N-methyl pyrolidinone, pyrolidinone, dimethylformamide, ordimethylsulfoxide.
- 23. (original) The composition of claim 1, further comprising a deoxyribonuclease inhibitor.
- 24. (original) The composition of claim 23, wherein the at least one deoxyribonuclease inhibitor comprises a divalent cation chelator.

- 25. (original) The composition of claim 24, wherein the chelator is EDTA, EGTA, of DPTA.
- 26. (withdrawn) A method for isolating nucleic acids from a biological sample comprising:

combining the sample with at least one cationic surfactant, at least one protease, and a buffer, to form a reaction composition;

incubating the reaction composition at a temperature suitable for releasing nucleic acid from the biological sample; and isolating the released nucleic acid.

- 27. (withdrawn) The method of claim 26, further comprising adding a second surfactant and a salt.
- 28. (withdrawn) The method of claim 27, wherein the second surfactant comprises a nonionic surfactant.
- 29. (withdrawn) The method of claim 28, wherein the nonionic surfactant is polyoxyethylene(20)sorbitan monolaurate (Tween 20), Tween 80, Triton X-100, Triton X-114, Triton X-305, Triton X-405, Brij-35, Brij-56, Brij-58, *n*-Decyl-β-D-glucopyranoside, *n*-Dodecyl-β-D-maltoside, *n*-Hexyl-β-D-glucopyranoside, *n*-Octyl-β-D-glucopyranoside, *n*-Tetradecyl-β-D-maltoside, alkyl glycosides, Glucamides, MEGA-10, MEGA-9, MEGA-8, Genapol X-80, Genapol X-10, Thesit, Lubrol PX, Genapol C-100, Pluronic F-127,

- APO-10, APO-12, Big CHAP, Digitonin, or polyethyleneglycol-*p*-isooctylphenyl ether (NP-40).
- 30. (withdrawn) The method of claim 27, wherein the second surfactant comprises Tween 20 and the salt comprises a sodium salt.
- 31. (withdrawn) The method of claim 26, wherein the biological sample comprises whole tissue.
- 32. (withdrawn) The method of claim 26, comprising the composition of claim 3.
- 33. (withdrawn) The method of claim 26, comprising the composition of claim 4.
- 34. (withdrawn) The method of claim 26, wherein the at least one cationic surfactant comprises cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTACI), hexadecyltrimethylammonium bromide, or hexadecyltrimethylammonium chloride, and the at least one protease comprises Proteinase K.
- 35. (withdrawn) The method of claim 26, further comprising exposing the released nucleic acid to at least one organic solvent.
- 36. (withdrawn) The method of claim 35, wherein exposing the nucleic acid to at least one organic solvent comprises extracting the nucleic acids, precipitating the nucleic acids, or both extracting and precipitating the nucleic acids.
- 37. (withdrawn) The method of claim 26, further comprising adding a solid phase component capable of binding the released nucleic acid.

- 38. (withdrawn) The method of claim 26, further comprising adding a chaotropic salt and at least one organic solvent to precipitate the released nucleic acid.
- 39. (withdrawn) The method of claim 38, wherein the at least one organic solvent comprises ethanol, isopropanol, or acetone.
- 40. (withdrawn) The method of claim 38, wherein the chaotropic salt comprises guanidinium thiocyanate, guanidine hydrochloride, sodium thiocyanate, sodium perchlorate, or sodium iodide.
- 41. (withdrawn) The method of claim 26, further comprising adding a polymer.
- 42. (withdrawn) The method of claim 41, wherein the polymer is a polyethylene glycol.
- 43. (withdrawn) The method of claim 26, further comprising adding a divalent cation capable of precipitating the nucleic acid.
- 44. (withdrawn) The method of claim 43, wherein the divalent cation is zinc.
- 45. (withdrawn) The method of claim 26, wherein the nucleic acid is ribonucleic acid.
- 46. (withdrawn) The method of claim 45, wherein the reaction composition further comprises a ribonuclease inhibitor.
- 47. (withdrawn) The method of claim 46, wherein the ribonuclease inhibitor comprises aurintricarboxylic acid, vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate,

bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenyhlic acid), zinc sulfate, bromopyruvic acid, formamide, copper, or zinc.

- 48. (withdrawn) The method of claim 47, wherein the ribonuclease inhibitor is aurintricarboxylic acid.
- 49. (withdrawn) The method of claim 45, wherein the reaction composition is incubated at a temperature of less than 60° C.
- 50. (withdrawn) The method of claim 49, wherein the reaction composition is incubated at a temperature between 40° C and 50° C.
- 51. (withdrawn) The method of claim 45, wherein the reaction composition has a pH of less than 8.0.
- 52. (withdrawn) The method of claim 51, wherein the reaction composition has a pH between 5.0 and 7.0.
- 53. (withdrawn) The method of claim 52, wherein the reaction composition is incubated at a temperature between 40° C and 50° C, and wherein the reaction composition further comprises aurintricarboxylic acid.
- 54. (withdrawn) A method for releasing nucleic acids from a biological sample comprising:

combining the sample with at least one cationic surfactant, at least one protease, and a buffer, to form a reaction composition; and

incubating the reaction composition at a temperature suitable for releasing the nucleic acids from the biological sample.

- 55. (withdrawn) The method of claim 54, further comprising adding a second surfactant and a salt.
- 56. (withdrawn) The method of claim 55, wherein the second surfactant comprises a nonionic surfactant.
- 57. (withdrawn) The method of claim 56, wherein the nonionic surfactant is polyoxyethylene(20)sorbitan monolaurate (Tween 20), Tween 80, Triton X-100, Triton X-114, Triton X-305, Triton X-405, Brij-35, Brij-56, Brij-58, *n*-Decyl-β-D-glucopyranoside, *n*-Dodecyl-β-D-maltoside, *n*-Hexyl-β-D-glucopyranoside, *n*-Octyl-β-D-glucopyranoside, *n*-Tetradecyl-β-D-maltoside, alkyl glycosides, Glucamides, MEGA-10, MEGA-9, MEGA-8, Genapol X-80, Genapol X-10, Thesit, Lubrol PX, Genapol C-100, Pluronic F-127, APO-10, APO-12, Big CHAP, Digitonin, or polyethyleneglycol-*p*-isooctylphenyl ether (NP-40).
- 58. (withdrawn) The method of claim 55, wherein the second surfactant comprises Tween 20 and the salt comprises a sodium salt.
- 59. (withdrawn) The method of claim 54, wherein the biological sample comprises whole tissue.
- 60. (withdrawn) The method of claim 54, comprising the composition of claim 3.
- 61. (withdrawn) The method of claim 54, comprising the composition of claim 4.

- 62. (withdrawn) The method of claim 54, wherein the at least one cationic surfactant comprises cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTACI), hexadecyltrimethylammonium bromide, or hexadecyltrimethylammonium chloride, and the at least one protease comprises Proteinase K.
- 63. (withdrawn) The method of claim 54, wherein the nucleic acid is ribonucleic acid.
- 64. (withdrawn) The method of claim 63, wherein the reaction composition further comprises a ribonuclease inhibitor.
- 65. (withdrawn) The method of claim 64, wherein the ribonuclease inhibitor comprises aurintricarboxylic acid, vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenyhlic acid), zinc sulfate, bromopyruvic acid, formamide, copper, or zinc.
- 66. (withdrawn) The method of claim 65, wherein the ribonuclease inhibitor is aurintricarboxylic acid.
- 67. (withdrawn) The method of claim 63, wherein the reaction composition is incubated at a temperature of less than 60° C.
- 68. (withdrawn) The method of claim 67, wherein the reaction composition is incubated at a temperature between 40° C and 50° C.
- 69. (withdrawn) The method of claim 63, wherein the reaction composition has a pH of less than 8.0.

- 70. (withdrawn) The method of claim 69, wherein the reaction composition has a pH between 5.0 and 7.0.
- 71. (withdrawn) The method of claim 70, wherein the reaction composition is incubated at a temperature between 40° C and 50° C, and wherein the reaction composition further comprises aurintricarboxylic acid.
- 72. (withdrawn) A kit for obtaining nucleic acid from a biological sample comprising at least one cationic surfactant and at least one protease.
- 73. (withdrawn) The kit of claim 72, comprising the at least one surfactant of claim 3.
- 74. (withdrawn) The kit of claim 73, wherein the at least one cationic surfactant comprises cetyltrimethylammonium bromide, cetyltrimethylammonium chloride, hexadecyltrimethylammonium bromide, or hexadecyltrimethylammonium chloride.
- 75. (withdrawn) The kit of claim 72, wherein the at least one protease is selected from the group consisting of subtilisins, subtilases and alkaline serine proteases.
- 76. (withdrawn) The kit of claim 75, wherein the at least one protease is Proteinase K.
- 77. (withdrawn) The kit of claim 72, further comprising a second surfactant and a salt.
- 78. (withdrawn) The kit of claim 72, further comprising at least one organic solvent for extracting the nucleic acids, precipitating the nucleic acids, or both extracting and precipitating the nucleic acids.

- 79. (withdrawn) The kit of claim 78, wherein the organic solvent for extracting nucleic acids comprises phenol and the organic solvent for precipitating nucleic acids comprises isopropanol or ethanol.
- 80. (withdrawn) The kit of claim 72, further comprising a solid phase component.
- 81. (withdrawn) The kit of claim 72, further comprising a chaotropic salt and an organic solvent.
- 82. (withdrawn) The kit of claim 72, further comprising a polymer.
- 83. (withdrawn) The kit of claim 72, further comprising a divalent cation capable of precipitating nucleic acid.
- 84. (withdrawn) The kit of claim 72, further comprising at least one ribonuclease inhibitor.
- 85. (withdrawn) The kit of claim 84, wherein the at least one ribonuclease inhibitor is aurintricarboxylic acid, vanadylate ribonucleoside complexes, phenylglyoxal, phydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, Bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenyhlic acid), zinc sulfate, bromopyruvic acid, formamide, copper, or zinc.
- 86. (withdrawn) The kit of claim 72, further comprising at least one solubilizing agent.
- 87. (withdrawn) The kit of claim 86, wherein the solubilizing agent comprises 1-methyl 2 pyrolidinone, N-methyl pyrolidinone, pyrolidinone, dimethylformamide, or dimethylsulfoxide.

- 88. (previously presented) A composition for macerating whole tissue, wherein the whole tissue is not blood, comprising: at least one cationic surfactant, at least one protease, a buffer, and the whole tissue.
- 89. (cancelled)
- 90. (currently amended) The composition of claim [[89]] <u>88</u>, wherein the at least one cationic surfactant is protonated under the conditions used.
- 91. (currently amended) The composition of claim [[89]] <u>88</u>, wherein the at least one cationic surfactant has the structure:

$$R_3$$
  $R_2$   $R_3$   $R_4$   $R_4$ 

wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are independently selected from the group consisting of: –H; an alkyl group comprising between one and twenty carbon atoms; and an aryl group comprising between six to twenty-six carbon atoms.

92. (previously presented) The composition of claim 91, wherein the cationic surfactant is an alkyltrimethyl ammonium salt, where R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are methyl

groups, and  $R_4$  is an alkyl group comprising 6, 8, 10, 12, 14, 16, or 18 carbon atoms.

- 93. (previously presented) The composition of claim 92, where the cation of the alkyltrimethyl ammonium salt is selected from the group consisting of cetyltrimethylammonium, hexadecyltrimethylammonium, tetradecyltrimethylammonium, dodecyltrimethylammonium, and lauryl trimethylammonium.
- 94. (previously presented) The composition of claim 92, where the anion (X<sup>-</sup>) of the alkyltrimethyl ammonium salt is selected from the group including bromide, chloride, iodide, hydroxide, nitrate, sulfate, phosphate, formate, acetate, propionate, oxalate, malonate, succinate, or citrate.
- 95. (previously presented) The composition of claim 91, wherein the at least one cationic surfactant is a benzyldimethyl-*n*-alkylammonium salt, where R<sub>1</sub> and R<sub>2</sub> are methyl groups, R<sub>3</sub> is an aryl group comprising six carbon atoms, and R<sub>4</sub> is an alkyl group comprising 6, 8, 10, 12, 14, 16, or 18 carbon atoms.
- 96. (previously presented) The composition of claim 95, where the anion of the benzyldimethyl-*n*-alkylammonium salt is selected from the group consisting of bromide, chloride, iodide, hydroxide, nitrate, sulfate, phosphate, formate, acetate, propionate, oxalate, malonate, succinate, and citrate.

- 97. (currently amended) The composition of claim [[89]] <u>88</u>, wherein the at least one protease is selected from the group consisting of subtilisins, subtilases, and alkaline serine proteases.
- 98. (previously presented) The composition of claim 97, wherein the at least one protease is selected from the group consisting of Proteinase K, Proteinase R, Proteinase T, Subtilisin DY, an alkaline serine protease from *Streptomyces griseus* or *Bacillus licheniformis*, Dispase, subtilisin Carlsberg, subtilopeptidase A, and thermolysin.
- 99. (previously presented) The composition of claim 97, wherein the protease is Proteinase K.
- 100. (previously presented) The composition of claim 97, wherein the protease is thermolysin or a thermostable protease.
- 101. (previously presented) The composition of claim 100, wherein the protease is from *Thermus* Rt41A or *Bacillus thermoproteolyticus rokko*.
- 102. (currently amended) The composition of claim [[89]] <u>88</u>, further comprising calcium chloride.

- 103. (currently amended) The composition of claim [[89]] 88, wherein the buffer maintains the pH between pH 7 and pH 9.
- 104. (currently amended) The composition of claim [[89]] <u>88</u>, wherein the buffer maintains the pH between pH 5 and pH 7.
- 105. (currently amended) The composition of claim [[89]] <u>88</u>, further comprising a ribonuclease inhibitor.
- 106. (previously presented) The composition of claim 105, where in the at least one ribonuclease inhibitor comprises aurintricarboxylic acid, vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenyhlic acid), zinc sulfate, bromopyruvic acid, formamide, copper, or zinc.
- 107. (previously presented) The composition of claim 105, wherein the at least one ribonuclease inhibitor comprises aurintricarboxylic acid.
- 108. (currently amended) The composition of claim [[89]] 88, wherein the cationic surfactant is cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTACI), hexadecyltrimethylammonium bromide, or hexadecyltrimethylammonium chloride; the protease is Proteinase K; the buffer

- maintains the pH between pH 5 and pH 7; and further comprising aurintricarboxylic acid.
- 109. (previously presented) The composition of claim 108, further comprising at least one solubilizing agent for enhancing the solubility or permeability of the sample.
- 110. (previously presented) The composition of claim 109, wherein the solubilizing agent is 1-methyl 2 pyrolidinone, N-methyl pyrolidinone, pyrolidinone, dimethylformamide, or dimethylsulfoxide.
- 111. (currently amended) The composition of claim [[89]] <u>88</u>, further comprising a deoxyribonuclease inhibitor.
- 112. (previously presented) The composition of claim 111, wherein the at least one deoxyribonuclease inhibitor comprises a divalent cation chelator.
- 113. (previously presented) The composition of claim 112, wherein the chelator is EDTA, EGTA, of DPTA.